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**EVALUATION OF FLAVORS
FOR MASKING SENSORY ATTRIBUTES
OF FISH OIL**



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April 1989

U.S. DEPARTMENT OF COMMERCE
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ABSTRACT

The objective of this present study was to determine the efficacy of flavors to mask the sensory attributes of fish oil so that adequate controls may be utilized for double blind clinical studies. The studies demonstrated two possible alternatives for providing adequate controls for conducting double blind studies with fish oil. The research design may choose to flavor the fish oil and control oil very strongly with a peppermint or lemon-lime flavor or to flavor the control oil with a mild, fish oil flavor. Both approaches were shown to be effective in producing a fish oil treatment that was indistinguishable from the control oils (corn oil, olive oil and safflower oil).

INTRODUCTION

Numerous human feeding trials have been prompted by the epidemiological observations of Bang et al. (1976) and Dyerberg (1975), which associated the beneficial effects of omega-3 (n-3) polyunsaturated fatty acids (PUFAs) with the potential prevention of coronary heart disease. In an effort to determine the mechanisms of n-3 PUFAs on cardiovascular function, volunteers have consumed fatty fish (Harris and Conner, 1980; Phillipson et al. 1985), fish oils (Fischer and Weber, 1984; Sanders et al. 1981), and fish oil concentrates (Sanders and Mistry, 1984; Harris et al. 1984) in a number of trials. During a review of n-3 feeding trials, Kinsella (1987) found several serious shortcomings. First, the experimental design used in many feeding trials did not include a control or comparison group. Secondly, many participants as well as the investigators were often aware of the treatment being used, which negated ascertaining the influence of a placebo. Also, few studies attempted to determine the amount of n-3 PUFA in the normal diet.

A conference held in 1985 titled "Health Effects of Polyunsaturated Fatty Acids in Seafoods" (Simopoulous et al. 1986) addressed several health areas such as thrombosis, atherosclerosis, immunology, inflammations, membrane function and metabolism. Recommendations from participants indicated that much more research is needed, especially clinical trials utilizing n-3 fatty acids. In particular, reference was made to the need for tasteless and odorless oils to carry out blind studies. The participants were also concerned with the nature of the placebo and its purity. Results of studies using impure placebos or those producing the same side-effects as the fish oil preparations may prove impossible to interpret. Leaf and Weber (1988) stated that if prospective, double-blind, placebo-controlled clinical trials were to show that n-3 fatty acids helped to prevent atherosclerosis, these agents would represent one of the most benign interventions in our pharmacopeia.

The purpose of blind trials is to reduce the observer and patient bias by concealing the identity of the treatment administered from either the patient or the physician (termed a single blind trial) or from both (termed a double blind trial). The efficacy of a particular treatment depends not only upon the actual pharmacological effects of a drug, but also on a number of non-specific factors termed a placebo effect (Spriet and Simon, 1985). In order to distinguish pharmacological effects from non-specific effects, the presumed active component may be compared to a placebo (a pseudo-drug identical in

appearance, but a pharmacologically inactive substance). The double blind studies involving fish oil commonly compare a test group treated with fish oil versus a control group treated with oral placebos of corn oil, safflower oil or olive oil. The first criteria in such a study is the need for an oral placebo which is identical in all respects to the active oral treatment except that the active ingredient is missing. Oils such as corn oil, safflower oil and olive oil are appropriate placebos to fish oil since they do not contain the long-chain n-3 polyunsaturated fatty acids eicosapentaenoic (C20:5n-3) and docosahexaenoic acid (C22:6n-3) prevalent in fish oil (Table 1). Other features required of a placebo in order to match the test treatment include color, texture, shape, size and mode of oral therapy (capsule, liquid, or tablet). While these oils are somewhat similar in texture, viscosity, and color, substantial differences exist in their sensory attributes of taste and odor. Since fish oil has a distinctive taste, the use of capsules has been the most feasible for masking taste, texture, and odor. However, the use of capsules do not totally obscure the treatments being given. An individual assigned to a fish oil treatment only has to bite into a capsule to realize which treatment he is receiving. Additionally, the ingestion of fish oil capsules often results in the repeating of a fishy flavor in a number of individuals. A study of fish oil and aspirin in restenosis produced mild gastrointestinal effects in seven patients in the fish oil group including belching, dyspepsia, and flatulence (Dehmer et al. 1988).

Of course, the importance and feasibility of conducting double-blind studies depends on the disease, the type of therapy, method of evaluation, and available resources. In those situations where the need exists for conducting double blind studies to independently assess the effects of fish oil and concentrates, it is desirable that the odor and flavor of fish oil be identical to the control treatment. There are few studies that have investigated the masking of fish oils, and none that have approached it from the aspect of matching placebos with fish oil. A study by Jellinik and Stansby (1971) explored the area of masking fish oils, but the objective in that study was to induce oxidation and evaluate flavors for masking rancid, oxidative flavors. The objective of the present study was to assess the use of flavors in high-quality fish oil and placebo oils in order to produce nondistinguishable treatments to facilitate the conduct of double blind clinical trials. The objective of this study, to determine the efficacy of flavors to mask the sensory attributes of fish oil, was addressed in the three experiments listed below.

METHODS

A partially refined menhaden oil (Brevoortia spp.) (Zapata Haynie¹, Reedville, VA) was vacuum deodorized by the U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service (NMFS), Charleston Laboratory, Charleston, SC and was used in all phases of testing. This treatment yielded a fish oil that had very little fishy flavor. The composition and quality of the fish oil as determined by quality assurance analysis (Van Dolah and Galloway, 1988) is given in Table 2. A sensory evaluation by a trained panel at the Charleston Laboratory is utilized to evaluate the acceptability of the fish oil products for human consumption (Gooch and Galloway, 1987). The flavor and odor attributes are based on a modification

of the Quantitative Descriptive Analysis method, whereby total intensity of flavor and odor components are ranked from absent to strong using an unstructured 15 cm scale (Stone et al. 1974). The scale is an unstructured 15 cm line with a vertical line at the left representing absence of flavor and a vertical line at the right reflecting very strong intensity of flavor. Standard oils are used to reference responses from absent or weak to very strong. Tocopherols and tertiary butylhydroquinone (TBHQ)(Kodak 5-67, Tenox GT-1, and Tenox 20A, Eastman Chem. Prod., TN) were added to the fish oil so that the final concentrations of antioxidants were: 1.0 mg/g each of alpha- and gamma-tocopherol and 0.2 mg/g TBHQ.

Both affective (i.e. preference) and analytical (i.e. difference) tests (Warner, 1985) were used with taste panels to evaluate the effectiveness of flavor masking agents. The study was carried out in three experiments after conducting a preliminary experiment to assess a panel consensus on flavor concentration. In experiment 1, affective testing by taste panels was used to determine the preference or acceptability of flavors in masking fish oil. Oil was stored at -40°C prior to use. Analytical testing was performed in experiments 2 and 3 to determine discriminative evaluation of flavors in masking the fish oil and placebo oils. These tests were conducted using the following techniques.

For all experiments, ten ml samples of vacuum deodorized menhaden oil were placed in glass culture tubes; flavors were added at the concentrations specified for each experiment, mixed well, refrigerated, and the taste panels were performed within a 5 day period. The trained panel (experiment 1, 2, 3) consisted of 5 people at the NMFS Charleston Laboratory and the small consumer panel (experiment 2) consisted of 11 people at Clemson University who were familiarized with testing procedures utilized with fish oil. The flavored test materials were evaluated at room temperature by taste panels. Disposable dispensing microliter pipets were used to transfer the test oil sample into the panelists' mouth. This procedure avoided coating the lips with oil and allowed small sample volumes (50-100 ul) to be tested. The panelists rinsed their mouths with warm water, if needed, between samples to revitalize the palate. In order to avoid sensory fatigue, no more than three samples were analyzed in one session except during the screening in experiment 1.

A preliminary experiment was performed to determine a consensus among the panelists as to an optimal concentration. To verify that the flavor concentrations were in the correct range for masking the fish oil, panelists were asked to evaluate various flavor concentrations for masking ability. Since the concentration of flavoring agents vary depending on the flavor itself and the manufacturer, it is important that the concentration used for masking are consistent among panelists. The flavors were prepared with the low concentration having poor masking, the medium concentration as achieving good masking, and the high concentration as overmasking. The panelists were asked to mark the concentration that allowed the best masking of the fish oil.

Table 1. Fatty Acid Composition¹ of Fish Oil, Olive Oil, Safflower Oil and Corn Oil.

FATTY ACID	FISH OIL (mg/g)	OLIVE OIL (mg/g)	SAFFLOWER OIL (mg/g)	CORN OIL (mg/g)
12:0	1.2	0.3	3.0	
14:0 ISO	0.2	0.1	2.2	
14:0	72.3	1.0		
14:1	0.3			
15:0 ISO	1.9	0.2		
15:0 AISO	0.7			
15:0	4.8			
16:0 ISO	0.8		64.0	102.1
16:0	148.6	90.6	64.0	102.1
16:1W11	2.1			
16:1W9	1.8	1.2	0.2	0.4
16:1W7	91.3	3.9	0.7	1.0
7MH	1.1			
16:1W5	2.5			
16:2W4	13.2			
16:3W4	16.6			
16:4W3/4W1	16.7			
16:2W7	2.3			
16:2W6?	0.6			
17:0	5.6	0.7	0.3	0.8
17:0 ISO	1.9			
17:0 AISO	0.8			
18:0 ISO	0.9			
18:0	26.6	27.1	24.9	18.7
18:1W9	70.2	603.1	137.8	244.2
18:1W7	26.0	13.8	5.9	6.5
18:1W?				1.3
18:1W5	0.9			
18:2W6	10.7	149.4	670.5	520.3
18:3W6	5.7	1.5		0.4
18:3W4	4.5	0.9		
18:3W3	9.7	7.4	1.0	11.5
18:4W3	26.1			
18:4W1	4.1			
20:0	2.4	4.2	3.2	
20:1		2.9		4.3
20:1W11	1.9			
20:1W9	11.6		2.1	
20:1W7	0.3			
20:1W5/NMID?	1.5			
NMID?	2.2			
20:2W6	1.4			
20:3W6	1.9			
20:4W6	8.9			
20:3W4?	0.8			
20:3W3?	0.9			
20:4W3	8.6			
20:5W3	135.4			
22:0	1.1	1.4	2.1	
22:1				1.5
22:1W11	1.2			
22:1W9	1.7			
22:1W7	0.3			
21:5W3	6.7			
22:4W6?	1.1			
22:5W6	2.6			
22:5W3	21.1			
22:6W3	82.7			
24:0	0.4	0.7	1.1	
24:1	2.5	0.1	1.3	1.6

¹ Analyses provided by the NMFS Charleston Laboratory Fish Oil Test Material Program.

Table 2. Composition and Quality of Vacuum-Deodorized Fish Oil Utilized in Flavor Studies.

ANALYSES ¹	FISH OIL
EPA, mg/g	135
DHA, mg/g	83
TOTAL N-3, mg/g	289
CHOLESTEROL, mg/g	3.3
FREE FATTY ACIDS, %	0.05
PEROXIDE VALUE, meq/kg	0.98
IODINE VALUE	183.6
ANISIDINE VALUE	35.54
ANTIOXIDANT CONTENT:	
a-TOCOPHEROL, mg/g	0.9
gamma-TOCOPHEROL, mg/g	0.8
TBHQ, mg/g	0.2
MOISTURE, ug/g	136
PCB (1254), ug/g	0.1
TOTAL DDT, ug/g ¹	0.02
SENSORY ATTRIBUTES:	
0-15; 15 MAX INTENSITY	
ODOR:	
TOTAL INTENSITY	3.28
BUTTERY	0
BEANY	0.06
RANCID	0
PAINTY	0.17
OXIDIZED	0.05
GRASSY	0.06
FISHY	1.29
BITTER	0
SWEET	0.8
FRUITY/MELON	0
BURNT	0
FLAVOR	
TOTAL INTENSITY	4.28
BUTTERY	0
BEANY	0.02
RANCID	0
PAINTY	0.24
OXIDIZED	0.2
GRASSY	0.06
FISHY	2.81
BITTER	0
SWEET	0
FRUITY/MELON	0
BURNT	0
PUTRID	0.41

¹ Total DDT = p,p-DDE + o,p-DDD + p,p-DDD

Experiment 1

This first experiment represented a screening of which flavors successfully masked the characteristic flavor of fish oil in a palatable manner. The flavors were chosen based on the literature, conversations with technical representatives of flavor companies, as well as personal opinions. Jellinik and Stansby (1971) found that lemon, root beer, wintergreen and cherry had promise in masking fish oil flavor. The following flavors and concentrations approved by the U.S. Food and Drug Administration for use in foods were chosen for evaluation as listed below in Table 3.

Table 3. Sources and Concentrations of Flavors Utilized in Experiment 1.

FLAVOR	IDENTIFICATION	CONCENTRATION
peppermint ¹	#H-9065	0.9%
cinnamon ²	#55701	0.6%
lemon ²	#55300	0.6%
orange ²	#55183	0.3%
lime ²	#55392	1.6%
cherry ²	#55153	0.8%
root beer ²	#55712	0.8%
almond ²	#55701	1.6%
lemon-lime ²	#55300; #55392	0.3%; 0.6%
coconut ³	#155	1.0%
pineapple ³	#176	0.4%
coconut-pineapple ³	#155; #176	1.0%; 1.0%

¹ Haarman and Reimer, Springfield, NJ

² Edlong Corporation, Elk Grove Village, IL

³ Lorann Oil, Inc., Lansing, MI

Samples of fish oil containing the flavor concentrations above were subjected to the following two taste panels: 1) an untrained panel at Clemson University Food Science Department and 2) a trained panel at the NMFS Charleston Laboratory. The panelists tested all 12 flavors during 1 session and were asked to rank the flavors (on a 1-10 scale; 10 being the best) and to choose the three flavors which they perceived were the most effective at masking the fish oil.

Experiment 2

On the basis of the flavors selected by the panelists in experiment 1, the following top three ranking flavors as well as the next two flavors were chosen to evaluate in a discriminative test with fish oils and placebo oils:

Experiment 1

The results obtained from experiment 1 appear in Table 5 below. The panelists ranked the flavors on a 1-10 scale and these results were summed to reflect the number of panelist choosing the flavor among the top three choices (i.e. peppermint was chosen among the top three flavors by 9 panelists from the consumer panel and 1 panelist from the trained panel). The flavors chosen by both the trained and consumer panels as the best top three were peppermint, cinnamon and lemon, followed by lemon-lime and root beer. The orange and cherry flavors may also provide potential masking agents for fish oil.

Table 5. Selection of Top Three Flavors Judged Most Acceptable in Masking Fish Oil by Taste Panels Conducted by a Consumer Panel at Clemson University Department of Food Science (n=11 panelists) and a Trained Panel at the National Marine Fisheries Service (NMFS) Charleston Laboratory (n=5).

FLAVOR	CLEMSON UNIVERSITY	CHARLESTON LABORATORY
peppermint	9	1
cinnamon	5	4
lemon	3	5
lemon-lime	7	0
root beer	1	4
orange	4	1
cherry	3	0
almond	1	0
coconut-pineapple	0	0
lime	0	0
coconut	0	0
pineapple	0	0

Experiment 2

The panelists were able to identify the fish oil in the lemon, cinnamon and root beer flavors (Table 6). In addition, some panelists were also able to identify some of the placebo oils. The flavors that showed the highest ability for masking were lemon-lime and peppermint, although one panelist reported that the peppermint was too strong and the olive oil placebo was detected by one panelist with the lemon-lime flavor. It should be noted that the flavor concentrations utilized in these experiments were different from those utilized in the preliminary experiment since new improved flavors were obtained from different flavor manufacturers.

Table 6. Results of Taste Panel Conducted at the NMFS Charleston Laboratory to Discriminate Between Flavored Placebo Oils and Fish Oil.

FLAVOR	CONCENTRATION	FISH	CORN	SAFFLOWER	OLIVE
Lemon-lime ¹	(0.3%; 0.6%) ³	0	0	0	+
Lemon ¹	(0.6%) ⁴	++++	0	0	+
Cinnamon ¹	(0.8%) ⁵	++++	+	0	0
Root beer ¹	(0.8%) ⁶	+++++	+	+	+++
Peppermint ²	(0.9%) ⁷	0	0	0	0

+ detected correct oil
 0 did not detect correct oil

Comments:
³ good masking, bitter
⁴ good overall result
⁵ too strong
⁶ poor masking
⁷ too strong

¹ Haarman and Reimer, Springfield, NJ
² Edlong Corp., Elk Grove Village, IL

Experiment 3

The panelists were unable to correctly identify any of the placebo oils when flavored with the fish flavor (Table 7). A + indicates a correctly identified oil while a 0 indicates an incorrect identification or inability to identify the oil by the panelists.

Table 7. Taste Panel Conducted at the NMFS Charleston Laboratory on Placebo Oils Flavored With Fish Flavor (n=5 panelists).

OIL	FISH FLAVOR CONCENTRATION	OIL IDENTIFICATION ¹
FISH OIL	0.0%	0 0 0 0 0
CORN OIL	0.4%	0 0 0 0 0
SAFFLOWER OIL	0.4%	0 0 0 0 0
OLIVE OIL	0.4%	0 0 0 0 0

¹ + indicates correct identification of the oil sample
 0 indicates incorrect identification of the oil sample
 Comments: was not as fishy as expected; very similar.

DISCUSSION

These experiments demonstrate that a vacuum-deodorized fish oil was effectively masked by two of the flavors tested, peppermint and lemon-lime. While panelists were not able to distinguish fish oils and placebo oils when utilizing peppermint and lemon-lime flavors, these flavors did require a high

concentration in order to mask the fish oil. Based on the results of experiment 2, a palatable flavor was difficult to achieve without using high flavor concentrations, which often resulted in a strong, bitter flavor. This may not be a problem when used in soft gelatin capsules. It is desirable to produce a placebo oil with flavor characteristics pleasing to a majority of individuals rather than merely producing a flavor slightly more desirable than fish oil. In this regard, it may be possible to decrease the flavor concentration needed to mask the fish oil by using a sweetener that would act to intensify the flavor and increase the palatability. Of course, one would have to be cautious that any additive agent would not cause interference with potential therapeutic effects. Efforts were made during this study to select flavoring agents which would not have any active components. It was determined from experiment 3 that it is feasible to reproduce the fish oil taste in placebo oils. Not one panel member was able to distinguish any of the placebo oils when flavored with salmon flavor from the vacuum-deodorized fish oil.

In many diseases there exists no effective standard treatment, so many times the control group in a randomized trial of new therapy remains untreated. The problem with this approach is that with control patients without treatment, one cannot decipher whether any response in the treated group is due to therapy or just to the act of being treated (i.e. patient attitude may be improved by the feeling that something is being done and the illness itself may improve). In any randomized trial of oral drug therapy versus untreated controls, it is worth considering treating the latter with a placebo (Pocock, 1983). It is well-recognized that placebo and active controls provide the most rigorous proof of effectiveness. Double-blind studies require considerable time and effort to ensure a successful study and the value of placebo controls cannot be underestimated. Sound practical evaluation of the effects of n-3 PUFAs cannot be attained without development of adequate controls. Thus far, controls utilized in n-3 PUFA research with fish oil and concentrates are limited to soft-gelatin capsules comprising placebo oils.

These studies demonstrate two possible alternatives for providing adequate controls for double blind studies utilizing fish oil. In producing controls similar in taste to the active component, the clinician involved in n-3 research may choose to flavor the fish oil and the control oil very strongly with a peppermint or lemon-lime or flavor the control oil with a mild, not unpleasant fish oil flavor. Both approaches were shown to be effective in producing a fish oil treatment that was indistinguishable from the control oils (corn oil, olive oil, and safflower oil). While these approaches were effective in masking the flavor and odor of the fish oil in the mouth, the after effects due to eructation were not evaluated. Proposed studies should test the rigor of the double blind aspect and whether subjects can detect the treatments after ingestion. There is a possibility that the fish oil flavor may be revealed in the flavored treatments thereby compromising the double blind nature of the study. If this does occur in the fish oil masked with flavors, it may be better to have the control treatment flavored with fish so that the treatments remain indistinguishable. There is a need for a more thorough assessment on the use of flavors in masking fish oil by larger consumer groups and the use of other additives such as sweeteners, particularly for commercial use. The fish oil and placebo oil might then be formulated into a number of dosage delivery systems including soft-gelatin capsules, microcapsules, and aerosol products.

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